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Research Article

Genetic Diversity Between and Within Sudanese Zebu Cattle Breeds Using Microsatellite Markers

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Abstract

The aim of the present study was to assess the genetic variation and establish the relationship between and within three Sudanese zebu cattle breeds using panel composed from 9 bovine specific microsatellite markers recommended by the International Society of Animal Genetics (ISAG). The study was performed on a total of 75 unrelated cattle individuals from Fuga, Kenana and Butana breeds. A total of 74 microsatellite alleles were identified with number of alleles at one locus ranging from 5 to 12 alleles. Sharing allele analysis showed no unique allele for any breed studied. High values for the observed heterozygosity were found all over the loci and the three breeds studied: Fuga (0.778); Butana (0.737) and Kenana (0.692). Moreover, gene diversity was also high for the 9 microsatellite studied in the

three breeds. Its overall value was 0.684 with values of 0.778, 0.737 and 0.692 for three breeds: Fuga, Butana and Kenana; respectively. Inbreeding values proved the absence of inbreeding between the three breeds as well as within breeds. Drawing phylogeny tree between the breeds prove that Butana and Kenana are within one cluster while Fuga is in another cluster, the three breeds are then coming from one ancestor. The observed high genetic diversity along with the high values observed for heterozygosity, in the three breeds studied, can be used in designing good programs for genetic improvement in Sudanese zebu cattle. This study reports on a comprehensive study of the genetic structure and diversity of Sudanese zebu cattle breeds. Significant amount of genetic variability in the three local Sudanese zebu cattle were observed. This genetic information revealed that Sudanese zebu cattle breeds constitute

important and diverse bank of genetic diversity for bovine breeding and conservation. The obtained genetic data shaded light on some issues related to the local Sudanese zebu cattle breeds origin and structure. The study proved that Sudanese zebu cattle breeds are important and viable targets for conservation for they display special traits both phenotypic and of cultural and historical nature that should earn conservation efforts.

Keywords: Zebu, microsatellite, Sudan, cattle, genetic diversity.

Introduction

It is well known that local breeds of cattle can play a vital role in relevant and sustainable livestock production in most Eastern

African if it is compared with exotic breeds; these local breeds are well adapted to survive and reproduce under the region's harsh environments (Okomo-Adhiambo, 2002). After the recent Sudan referendum, the population of North Sudanese cattle was estimated around 17.465 million heads (Saeed, 2010). In Sudan there are many local cattle breeds including zebu and taurine species. Some authors tried to classify the Sudanese local cattle breeds on the basis of their origin and phenotypic characteristics. The Sudanese local cattle breeds were classified by Bennett *et al.* (1948) into three main groups; namely, Northern or Arab, Southern or Nilotic and the small cattle of the Nuba mountains. Most of the Sudanese cattle are from the Zebu cattle (Bennett *et al.,* 1954), the Sudanese Kenana and Butana cattle breeds are part of the Large East African Zebu group (*Bos indicus*) descended from the zebu introduced into Africa from West Asia. Available

archaeological records indicate that they are the most recent types of cattle to be introduced into Africa (Marshall, 2000). According to Joshi *et al.* (1957) and Payne (1970), the Northern Sudan cattle include Kenana, Butana, Western Baggara, White Nile and Northern Provinces. Other types of Northern Sudan Zebu cattle include Ayrashai (of eastern Sudan), Fuga or Dar El Reeh cattle of the North Kordofan, which is also from the zebu type (WSRMP Livestock Breed Characterizations Study, 2011). However, these classifications are based on phenotypic characteristics or geographic origin and are not related to genotype except in as much as the phenotype is in part a reflection of genotype. With the advent of molecular biology technology, a powerful new tool is available for characterization, classification and estimation of distances between breeds and strains.

The investigation of genetic variation is very important for future monitoring of gene flow in populations, conservation of species, determination of the level of inbreeding and crossbreeding within and between breeds (Hetzel and Drinkwater, 1992; Kunene *et al.,* 2007). In the last decade, microsatellite markers were extensively used to determine the genetic diversity and relationships among cattle breeds that has been documented in many studies (Rogić *et al.,* 2011; Medugorac *et al.,* 2009 Jordana *et al.,* 2003; Metta *et al.,* 2004; Mukesh *et al.,* 2004). Since microsatellite markers are co-dominant and multi-allelic attributes, they prove to be efficient in genetic diversity studies, and had become the most markers of choice in characterization of cattle breeds (Rehman and Khan 2009; Edwards *et al.,* 2000; Canon *et al.,* 2001). To our best knowledge, there are no previous studies on microsatellites polymorphism in cattle raised in

Sudan. The present study was carried out for employing the microsatellite polymorphisms in three different Sudanese cattle breeds: Fuga, Butana, and Kenana, for identifying the genetic relationship within and between these three breeds, inbreeding measurements, determining the purity of these breeds, finally calculating the genetic distance and drawing the phylogenic tree between these breeds.

Materials and Methods

Blood Samples and DNA Extraction

Ninety blood samples were collected from three different regions representing the three cattle breeds under study, randomly selected pure adult breed: Dar el Reeh (Fuga); Butana and

Kenana. The blood sample was collected on a tube supplied with 0.5 ml of 0.5 M EDTA (as an anticoagulant). Bovine genomic DNA was isolated and purified using phenol-chloroform and ethanol precipitation (Sambrook *et al.,* 1989). DNA concentration was determined using a UV spectrophotometer at optical density of 260 nm.

Microsatellite Analysis

Commercial one PCR multiplex (Bovine Genotypes™ Panel 1.2, F-904), obtained from Finnzyme Company (Finland), consists of nine fluorescence-labeled microsatellite primers were used for the analysis. The multiplex contained the microsatellites: ETH10, ETH225, BMC1824, BMC2113, SPS115, TGLA122, TGLA126, TGLA227, INRA23, the multiplex is under the recommendation of

ISAG (2012). For amplification, 100 ng of genomic DNA was added to a reaction mixture containing 50 pMol of fluorescence-labeled forward and reverse primers; 200 µM of every dNTPs; 1.5 mM of MgCl2 and 0.5U of Taq polymerase in a final volume of 25 µl. The amplification procedure was: initial denaturation step of 1 min at 95°C, 35 cycles of 1 min at 95°C, annealing 1 min at 57°C and 1 min at 72°C and a final extension of 5 min at 72°C. Amplicons obtained by PCR were separated by electrophoresis on an ABI 3730 instrument (Applied Biosystems) according to manufacturer recommendations and allele sizing was accomplished by using the internal size standards GeneScan 500 LIZ (Applied Biosystems). Allele nomenclature followed was that recommended by the Cattle Molecular Markers and Parentage Testing Workshop at the International Society of animal genetics (ISAG) Conference of Cairns in 2012.

Statistical Analysis

For calculating the allele frequencies, observed number of alleles, effective number of alleles (Kimura and Crow, 1964). Observed (Ho) and expected (He) heterozygosity at each locus in the three populations under study, polymorphism information content (PIC) value for each locus was calculated by using the method of Bostein et al. (1980). Pair-wise sharing alleles were calculated manually from the raw results using the variance-base method described by Weir and Cockerham (1980). All the previous calculations were in a software package called POPGENE which is developed by Yeh et al. (1999). Fisher statistics for population differentiation was computed using FSTAT version 2.9.3.2 computer program (Goudet, 2002). The calculated parameters included: mean a standard deviations of the F-statistics program,

F .f, that are analogue to Wright's (1951, 1978). Inbreeding estimates within the same breed (Fis) and between breeds (Fst) were obtained across breeds by the Jackknifing procedure over loci (Weir, 1990). The island model under neutrality and negligible mutation proposed by Slatkin (1985) was used to calculate the effect of migration and gene flow on the genetic structure of the analyzed populations. Calculations proposed by Nei et al. (1972) were used to identify genetic distances among populations, using (Ds) standard genetic distance and the DA distance of Nei et al. (1983).

Results

In the present study nine bovine microsatellites markers: BM1824, BM2113, INRA023, SPS115, TGLA122, TGLA126,

TGLA227, ETH10, ETH225 were analyzed in three different breeds of cattle found in Sudan (Fuga, Butana, and Kenana). TGLA122 presenting the highest number of allele per locus (12), while BM1824 presented the lowest (5) number of alleles. The results regarding the numbers of shared alleles between the different populations under study are presented in Table (1).The mean number of alleles shared between Fuga and Butana is 4.4, between Fuga and Kenana is 8.6 and between Butana and Kenana is 4, whereas the mean number of the alleles shared by the three breeds is 3.8. Except for the marker INRA023 and TGLA126 which gave only 4 and 5 alleles, respectively present in all the populations, the allele sharing results did not show any obvious results, unique or specific alleles for specific region or population.

Please see table 1 in the PDF version

The estimated parameters correlated to genetic polymorphism in three Sudanese zebu cattle breeds *viz.,* observed and effective numbers of alleles, heterozygosity (observed and expected) are presented in Table (2). Reasonable amount of variability in the three studied breeds was clearly observed from the allele frequency data. A total of 74 alleles were detected across the 9 loci with an average of 7, 5 and 5 alleles per locus (mean number of alleles in Fuga, Butana and Kenana breeds, respectively). The number of observed alleles ranged from 5 at locus BMC1824 to the highest of 9 alleles at loci TGLA 122 in Fuga, and 4 (BMC1824, ETH10, SPS115) to 6 (TGLA 122, TGLA 126, TGLA 227) in Butana and 4 (BMC2113, ETH10) to 8 (TGLA 122) in Kenana

Please see table 2 in the PDF version

The highest mean effective number of alleles (3.963) was observed in Fuga cattle when compared with the Butana (3.307) and Kenana (3.123) breeds. The Ne values were in range of 5.867 (INRA23) to 1.844 (SPS115) in Fuga and 2.142 (SPS115) to 4.769 (TGLA 126) Butana cattle and 1.469 (ETH225) to 4.420 (BMC1824) in Kenana. The observed mean (Ho) and expected (He) heterozygosity were 0.778 and 0.725 in Fuga vs. 0.737 and 0.695 in Butana and 0.693 and 0.651 in Kenana cattle, respectively. The difference between the observed and expected values (chi- square) was highly significant at $p < 0.01$ for all the markers in all the populations studied. The values of Polymorphism Information Content (PIC) varied from 0.304 (ETH225) in Kenana to 0.793 (BMC3113) in Fuga breed. The overall mean values of (PIC) obtained in the present study were 0.664 in Fuga, 0.630 in Butana and 0.596 in Kenana.

Concerning the results of the gene diversity for the nine microsatellites in the three breeds studied, the results are presented in Table (3). The average gene diversity over all loci were 0.684, while for individual loci the average gene diversity ranged between 0.461 (ETH10) in Kenana breed and 0.885 (TGLA122) in Fuga breed.

Please see table 3 in the PDF version

Results of *F*-statistics for each of the nine loci across breeds are presented in Table (4). The global deficit of heterozygotes across populations (Fit) amounted to 0.1%. An overall mean of deficit of heterozygotes (Fis) is -0.091. The overall genetic differentiation among breeds (Fst) was moderate (8.4%) but highly significantly different from zero. The highest Fst values were found for

SPS115 (0.235), TGLA122 (0.113), TGLA227 (0.105). Estimates of gene flow (Nm) value indicate a high rate of genetic flow between the populations (2.714).

Please see table 4 in the PDF version

A further breakdown of within-the breeds inbreeding estimates {Fixation index statistics (Fis = f)} at each microsatellite locus in the three Sudanese breeds under study are presented at Table (5). It is observed that the lowest Fis value was found in Butana (-0.830) as compared with Kenana (-0.195) and Fuga (-0.317)

Please see table 5 in the PDF version

Estimation of the divergence time for three breeds is presented at Table (6). Estimation of Nei's standard genetic distances (Ds) and assumed mutation rates of microsatellites loci (α) were used to estimate the time of divergence (t, in generations) Where, $Ds= 2\alpha t$.The Ds method described by Nei (1972) for determining genetic distances was used. Genetic distance measures the time that has elapsed since populations were genetically equivalent. The results demonstrated that the biggest divergence time (1407 years) was between the Fuga and Butana cattle; in contrast the lowest divergence time (343 years) was between Butana and Kenana.

Please see table 6 in the PDF version

Genetic distance matrix declared that the highest genetic distance was found between Fuga and Butana breeds (0.482). The lowest value for genetic distance was found between Kenana and Butana (0.118) (Table 7).

Please see table 7 in the PDF version

High values for genetic identity means low values for genetic distance and vice versa. The Dendrogram is based on Nei's (1972) using Genetic distance: Method = UPGMA (computer software), modified from NEIGHBOR procedure of PHYLIP Version 3.5 was used to draw the phylogeny tree between the three breeds understudy. The dendrogram showed that the Butana and Kenana are within one cluster while Fuga is in another cluster, (Fig 1).

Please see figure1 in the PDF version

Discussion

The sustainability of species and populations in the future is affected by the genetic diversity which shaped the past populations process (Soule, 1987). Maintaining of genetic diversity is a key to the long-term survival of most species including cattle (Hall and Bradley 1995). Many studies proved that farm animal genetic diversity is needed to meet current production requirements to allow sustained genetic improvement and to facilitate the rapid adaptation to changing breeding goals (Hall and Bradley 1995; Kumar *et al.* 2006). Diversity can be defined as the genetic variation between and within different breeds, so it is essential to characterize a breed

for its conservation. Microsatellites markers are the best genetic marker have been used successfully to define genetic structures and genetic relationships among different breeds. Microsatellites usually show higher numbers of alleles and subsequently polymorphism. Consequently, they enable population differentiation to be found more efficiently. Microsatellites markers especially autosomal had been the most used genetic markers to estimate genetic diversity and to investigate different breed relationships moreover to define conservation priorities (Lenstra *et al.,* 2012).
Neutral genetic diversity preservation is expected to contribute to maintaining specific breed traits due to natural and manmade selection.

Indeed, some microsatellites can be present in genes associated with important quantitative traits loci (QTLs) including adaptation (Hall *et al.,* 2012).
Previous studies have been performed concerning genetic diversity and relationship between three local cattle populations (Gangatiri, Shahabadi and Purnea) and two established cattle breeds (Bachaur and Siri) of eastern India by using 21 FAO and ISAG recommended microsatellite markers (Sharma et al., 2013). In a study conducted by Rehman and Khan (2009) for identification the genetic diversity of Hariana and Hissar Pakistani cattle breeds using 30 bovine microsatellite markers suggested by a joint committee of the Food and Agriculture Organization and the International Society for Animal Genetics. However, no information is available on gene differentiation among different cattle breeds raised in Sudan. In the present

study, genetic variation within and between three Sudanese cattle breeds named: Fuga, Butana, and Kenana were estimated using genotypic data of 9 microsatellite markers recommended by ISAG (2012) for such studies. The total numbers of animals genotyped were 75 animals, 25 animals from each breed. Out of the 9 microsatellite loci, 74 loci amplified successfully and produced definite banding patterns. Since it is observed a large numbers of alleles for these microsatellite markers, these markers could be fruitfully used in further studies on quantitative trait loci (QTL) detection and subsequently marker assisted selection (MAS). However, the allele sharing results did not show any obvious unique or specific alleles for specific breed. This is may come from the lack of breeding programs or in another words the absence of selection for genetic improvement.

The average of observed allele number was 8 alleles; this number lies within the range of 6-9 alleles, which was reported in many cattle breeds from Europe (MacHugh *et al.,* 1997, 1998), Africa (MacHugh *et al.,* 1997; Ibeagha-Awemu *et al.,* 2004); Brazil (Egito *et al.,* 2007). From another side, the observed average allele number is less than that reported for Indian zebu cattle, which is ranging from 4-16 (Mukesh *et al., 2004*; Chaudhari *et al.,* 2009, Sodhi *et al.,* 2011). This may be due to the large number of cattle breeds raised in India and the microsatellite used in the study, some microsatellites can produce more allele than others. The observed number of alleles demonstrated that almost all the microsatellite loci utilized in the present study were sufficiently polymorphic. All breeds showed that by the increase of number of alleles at different loci, there was an increase in mean genetic diversity in population and supported by Moioli *et al.* (2001).

This is an indication for the high ratio of heterozygosity which arises from the absence or weak selection or organized breeding programs for the Sudanese cattle. The effective number of alleles (Ne) can be identified as an estimate for the number of alleles with equal frequencies corresponding to a particular PIC value. Fuga cattle have the highest mean effective number of alleles (3.963) when compared with the Butana (3.307) and Kenana (3.123) breeds. The observed mean (Ho) and expected (He) heterozygosity were 0.778 and 0.725 in Fuga vs. 0.737 and 0.695 in Butana and 0.693 and 0.651 in Kenana cattle, respectively. In all the three breeds studied and for all the markers used, there were few individuals carrying homozygous alleles. Accordingly the values of the expected heterozygosity were very high for all the markers and populations under study.

The values of observed heterozygosity were higher than the expected heterozygosity indicates much of variability.
The Polymorphism Information Content (PIC) is an expected heterozygosity derived from allele frequencies in random mating populations. PIC is an indicator of how many alleles a certain marker has how much these alleles divided evenly. For example if a marker has many alleles but only one of them is frequent, the PIC will be low. The overall mean values of (PIC) obtained in the present study were 0.664 in Fuga, 0.630 in Butana and 0.596 in Kenana. While The average gene diversity over all loci were 0.684 that is almost similar to the previously reported by Loftus et al. (2002), which was 0.78 during their study concerning the identification of zebu alleles in some cattle breeds. There was a significant positive relationship between averages within population gene diversity for each locus. Kalinowski (2002)

observed high values of (PIC) and attributed it to the large number of alleles or heterozygosity. The observed high number of alleles may be attributed to the absence of selection pressure used for the improvement of draught characters. These findings are in agreement with Muralidhar (2003), who used ten microsatellite markers and obtained PIC values in Indian cattle which ranged from 0.150 to 0.790 in Ongole cattle breed and from 0.13 to 0.80 in Deoni cattle breed. Moreover, Rehman and Khan (2009) demonstrated that the value of PIC was 0.749 in Hariana and 0.719 in Hissar cattle. Higher PIC values were also seen in the Brazilian and Indian zebu cattle investigated earlier using microsatellite markers (Egito *et al.,* 2007; Pandey *et al.,* 2006; Kale *et al.,* 2010 and Sodhi *et al.,* 2011).

According to Holsinger and Weir (2009), Wright's F-statistics provide important insights into evolutionary processes that

influence the structure of genetic variation within and between populations, for that they are most widely used descriptive statistics in population and evolutionary genetics. Hart and Clark (1997), measures the heterozygote deficit relative to its expectation under HWE (Fst). Regarding the interpretation of fixation index (Fst), it had been accepted that a value ranging between 0 to 0.05 indicates low genetic differentiation; a value ranging between 0.05 and 0.15, medium differentiation; a value ranging between 0.15 and 0.25 big differentiation; and a value above 0.25, very big genetic differentiation (Wright, 1978; Balloux and Lugon-Moulin, 2002). Accordingly in our study Moderate genetic differentiation (Fst) among breeds (8.4%) implies that 91.6% of the total genetic variation corresponds to differences among individuals. In addition, a very low inbreeding rates (Fit= 0.1%) between the three breeds was detected that

means absence of inbreeding between the populations under study.
Genetic differentiation of similar magnitude has been reported among 12 African *Bos indicus* and *Bos taurus* cattle breeds (Ibeagha-Awemu and Erhardt, 2005). However, Figures is higher than the 7 % of the total genetic variability (mean FST=0.07) reported by Canon *et al.* (2001) among local European cattle breeds and much more higher than the 1.6% given by Ibeagha-Awemu and Erhardt (2006) among Red Bororo and White Fulani cattle breeds of Nigeria and Cameroon. However, the same value was found among 12 African *Bos indicus* and *Bos taurus* cattle breeds (Ibeagha-Awemu and Erhardt, 2005).
In this study Fst value may indicate the presence of gene flow between cattle breeds. The highest gene flow between breeds was found in the marker INRA023 (8.8508), while the lowest

gene flow was shown in the marker SPS115 (0.814). On the other hand, the presence of gene flow between these breeds may be due to their common origin (Canon *et al.,* 2000).
The inbreeding estimates were calculated using the FIS values (Wright's Fixation Index). This revealed that Sudanese breeds are having wider genetic variability. It is observed that the lowest Fis value was found in Butana (-0.830) as compared with Kenana (-0.195) and Fuga (-0.317) with an overall mean of deficit of heterozygotes (Fis) is (-0.091). This negative mean value 0.091 suggests that 9.1% of heterozygous excess individuals available in the breed and the samples were collected from highly heterozygous breed. This high heterozygosity values are comparable with Umblachery cattle breed (-0.0487) (Karthickeyan *et al.,* 2007). In contrast to our results, Metta (2004) reported in Indian Ongole cattle breed a high Fis values

(0.36) and the author attribute this high value to the small sample size studied (n=17). Similar results were obtained by Sharma *et al.,* (2006) in their study on Indian Bachaur cattle breed (Fis=0.22) and Sharma *et al.,* (2007) in Indian Gangatiri cattle breed (Fis=0.31). The estimated time of divergence revealed that the biggest divergence time (1407 years) was between the Fuga and Butana cattle; in contrast the lowest divergence time (343 years) was between Butana and Kenana. These results are confirming the phylogeny dendrogram obtained using UPGMA method that proved that Butana and Kenana are within one cluster while Fuga is in another cluster; the three breeds are then coming from one ancestor. This result could be logic due to raising of both the Kenana and Butana cattle in near or close areas as they raised in north of Sudan while Fog

were raised in the North Kordofan (Yousif and Fadl El- Moula, 2006).
In conclusion, this study reports on a comprehensive study of the genetic structure and diversity of three native zebu cattle breeds in Sudan. The genetic analysis data showed that a significant amount of genetic variation is maintained in the three studied Sudanese local zebu cattle breeds and all breeds studied could be considered as distinct genetic content. The three breeds displayed a markedly higher allelic richness most likely as a result of a combination of natural selection in diverse environmental conditions. Several authors declared that the amount and distribution of genetic diversity should be taken into account when dealing with conservation strategies of livestock species. It should be also taken into consideration that cultural, historical, and traditional aspects regarding the use of particular

breeds are relevant issues. Moreover, it should be realized the fact that directional selection for genetic improvement achieved by animal breeders has shaped animal genomes in unexpected ways through choosing the good or favorite alleles or genes structures for which the surrogate neutral markers used in diversity surveys are not necessarily fully representative.

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